against a variety of Brucella species. The inventive protein is effective at eliciting immune response.

The protein described herein measures antibodies against proteins of the organism. Diagnostic probes based on the nucleotide sequence of the 28 kDa antigen can be made.

Finally, since the protein gives rise to immune response, the protein may be used as a subunit vaccine for brucellosis. Compositions containing the subject proteins or polypeptides of the protein in pharmaceutically acceptable carriers are useful as vaccines and as diagnostic agents to identify protective antibodies. The proteins may be attached to solid supports for use in diagnostic studies known in the art.

Both the 28 kDA protein and polypeptides containing over 90 amino acid peptides of the protein thereof, which are reactive with antibodies raised to brucella species, can be may be administered by mouth. Antigenic fusion proteins containing sequences of other proteins such as cholera subunits are useful for administration orally or to the mucosa (for example intra-nasally). The fusion proteins may be lyophilized and inhaled from a vial for administration.

Materials and Methods:

A genomic library of B. melitensis DNA was generated in lambda gt11 obtained from Promega Corp., Madison, Wisconsin. The bacterial genomic DNA was digested with HaeIII to generate fragments with an average size of 2.5 kilo base-pairs (kb). The DNA was then methylated with EcoRI methylase followed by addition of EcoRI linkers and digestion with EcoRI restriction enzyme.

sequence of interest shown below:

ccccctgacataacccgctttgtccaaatttttttcaacttttcctgtaggagattttatga
acactcgtgctagcaattttctcgcagcctcatttccacaatcatgctcgtcggcgctt
tcagcctgcccgctttcgcacaggagaatcagatgacgacgcagcccgcgcgcatcgccg
tcaccggggaaggcatgatgacggcctcgcccgatatggccattctcaatctctcggtgc
tacgccaggcaaagaccgcgcgcgaagccatgaccgcgaataatgaagccatgacaaaag
tgctcgatgccatgaagaaggccggcatcgaagatcgcgatctccagacaggcggcatca
atatccagccgatttatgtctatcctgacgacaagaacaacctgaaagagcctaccatca
ccggctattctgtatccaccagtctcacggttcgcgtgcgcgaactggccaatgttggaa
aaattttggatgaatccgtcacgctcggtgttaatcagggcggtgatttgaacctggtca
atgataatccctccgccgtgatcaacgaggcgcgcaagcgcgcagtggccaatgccattg
ccaaggcgaagacgcttgccgacgctgcaggcgtggggcttggccgtgtggtggaaatca
gtgaactgagccgcccgcccatgccgatgccaattgcgcgcggacagttcagaaccatgc
tagcagccgcaccggacaattccgtgccgattgccgcaggcgaaaacagctataacgtat
cggtcaatgtcgttttgaaatcaagtaaatagctggggtatgacgcccttgccacctg
atacaaaacgccggcctggtttcacaggccggtttttttgattagagcgcgtttcgatct
gattgaatccgatcggcgctctaatcctttgttttgacgcgcatcttttccgaaaaccgt
ttcacactttcgggatgcggtctagcggatgatcgggcaaccgcgcgtatcggcaaatg
tcacg (Seq. ID No. 1)

A database search of the National Center for Biotechnology Information database was performed. However, no significant homologies were found. The gene encodes the 28 kDa antigen contained within the genomic material of Brucella species. The DNA/amino acid sequence was identified to be:

```
      1
ccccctgacataacccgctttgtccaaatttttttcaactttttcctgtaggagattttatga   60
      1                                                        M  N   20

acactcgtgctagcaattttctcgcagcctcattttccacaatcatgctcgtcggcgctt     120
  T  R  A  S  N  F  L  A  A  S  F  S  T  I  M  L  V  G  A  F      40

tcagcctgcccgctttcgcacaggagaatcagatgacgacgcagcccgcgcgcatcgccg     180
  S  L  P  A  F  A  Q  E  N  Q  M  T  T  Q  P  A  R  I  A  V      60

tcaccggggaaggcatgatgacggcctcgcccgatatggccattctcaatctctcggtgc     240
  T  G  E  G  M  M  T  A  S  P  D  M  A  I  L  N  L  S  V  L      80

tacgccaggcaaagaccgcgcgcgaagccatgaccgcgaataatgaagccatgacaaaag     300
  R  Q  A  K  T  A  R  E  A  M  T  A  N  N  E  A  M  T  K  V     100

tgctcgatgccatgaagaaggccggcatcgaagatcgcgatctccagacaggcggcatca     360
  L  D  A  M  K  K  A  G  I  E  D  R  D  L  Q  T  G  G  I  N     120

atatccagccgatttatgtctatcctgacgacaagaacaacctgaaagagcctaccatca     420
  I  Q  P  I  Y  V  Y  P  D  D  K  N  N  L  K  E  P  T  I  T     140

ccggctattctgtatccaccagtctcacggttcgcgtgcgcgaactggccaatgttggaa     480
  G  Y  S  V  S  T  S  L  T  V  R  V  R  E  L  A  N  V  G  K     160

aaattttggatgaatccgtcacgctcggtgttaatcagggcggtgatttgaacctggtca     540
  I  L  D  E  S  V  T  L  G  V  N  Q  G  G  D  L  N  L  V  N     180

atgataatccctccgccgtgatcaacgaggcgcgcaagcgcgcagtggccaatgccattg     600
  D  N  P  S  A  V  I  N  E  A  R  K  R  A  V  A  N  A  I  A     200

ccaaggcgaagacgcttgccgacgctgcaggcgtggggcttggccgtgtggtggaaatca     660
  K  A  K  T  L  A  D  A  A  G  V  G  L  G  R  V  V  E  I  S     220

gtgaactgagccgcccgcccatgccgatgccaattgcgcgcggacagttcagaaccatgc     720
  E  L  S  R  P  P  M  P  M  P  I  A  R  G  Q  F  R  T  M  L     240

tagcagccgcaccggacaattccgtgccgattgccgcaggcgaaaacagctataacgtat     780
  A  A  A  P  D  N  S  V  P  I  A  A  G  E  N  S  Y  N  V  S     260

cggtcaatgtcgtttttgaaatcaagtaaatagctggggtatgacgcccttgccacctg      840
  V  N  V  V  F  E  I  K  *                                       280

atacaaaacgccggcctggtttcacaggccggtttttttgattagagcgcgtttcgatct     900

gattgaatccgatcggcgctctaatcctttgttttgacgcgcatcttttccgaaaaccgt     960

ttcacacttttcgggatgcggtctagcggatgatcgggcaaccgcgcgtatcggcaaatg    1020

tcacg  1025 (Seq. ID No. 1 (DNA) and Seq. ID No. 2 (protein))
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Southern blots with a 28 kDa specific probe revealed that the gene is contained within a 8.5 kb EcoRI fragment in B. melitensis 16 M and Rev1, B. abortus 2308 and S19, B. suis, B. neotome, B. Canis and B. ovis.

The 28 kDa Brucella protein was shown to have the sequence: Met Asn Thr Arg Ala Ser Asn Phe Leu Ala Ala Ser Phe Ser Thr Ile Met Leu Val Gly Ala Phe Ser Leu Pro Ala Phe Ala Gln Glu Asn Gln Met Thr Thr Gln Pro Ala Arg Ile Ala Val Thr Gly Glu Gly Met Met Thr Ala Ser Pro Asp Met Ala Ile Leu Asn Leu Ser Val Leu Arg Gln Ala Lys Thr Ala Arg Glu Ala Met Thr Ala Asn Asn Glu Ala Met Thr Lys Val Leu Asp Ala Met Lys Lys Ala Gly Ile Glu Asp Arg Asp Leu Gln Thr Gly Gly Ile Asn Ile Gln Pro Ile Tyr Val Tyr Pro Asp Asp Lys Asn Asn Leu Lys Glu Pro Thr Ile Thr Gly Tyr Ser Val Ser Thr Ser Leu Thr Val Arg Val Arg Glu Leu Ala Asn Val Gly Lys Ile Leu Asp Glu Ser Val Thr Leu Gly Val Asn Gln Gly Gly Asp Leu Asn Leu Val Asn Asp Asn Pro Ser Ala Val Ile Asn Glu Ala Arg Lys Arg Ala Val Ala Asn Ala Ile Ala Lys Ala Lys Thr Leu Ala Asp Ala Ala Gly Val Gly Leu Gly Arg Val Val Glu Ile Ser Glu Leu Ser Arg Pro Pro Met Pro Met Pro Ile Ala Arg Gly Gln Phe Arg Thr Met Leu Ala Ala Ala Pro Asp Asn Ser Val Pro Ile Ala Ala Gly Glu Asn Ser Tyr Asn Val Ser Val Asn Val Val Phe Glu Ile Lys (Seq. ID No. 2)

The 28 kDa protein is obtained from lysates of the cell cultures and is purified by ion exchange to apparent homogeneity. Other methods known in the art appropriate for use include reverse phase HPLC and size exclusion chromatography.

Western blots of whole cell extracts of E. coli containing

the immunogenic peptides therefrom.

Immunogens are prepared by exposing the lysate to gel electrophoresis, then excising the 28 kDa bands from gel. The 28 kDa-containing band is then subjected to electrophoretic purification. The bands are pulverized in liquid nitrogen, then mixed with complete adjuvant and injected subcutaneously into the rabbits. The animals are given boosters after two weeks. Three to four days after the booster injection, the sera containing polyclonal antibodies is collected and screened. Serum containing antibodies can be detected by Western-blot.

Antibodies prepared against the 28 kDa antigen may be used to identify the infectious organisms in body fluids of mammals suspected of having brucellosis. Activity of monoclonal and polyclonal antibodies against 28 kDa antigens can be tested by several means. Western blot is used for the initial screening using 28 kDa bands as electrophoretically transferred to nitrocellulose paper. This screening procedure selects for high affinity antibodies, which are bound to the antigenic protein, since they must survive stringent washing methods. The monoclonal antibodies are used in screening the cDNA libraries. ELISA methodology can also be used as an alternate for initial screening by detecting binding of antigenic 28 kDa protein to antibodies.